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Determination of β-Globin Gene Cluster Haplotypes and Prevalence of α-Thalassemia in Sickle Cell Anemia Patients in Venezuela

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Sickle cell anemia and α-thalassemia have a heterogeneous distribution in Venezuela with a high frequency in the coastal area (sea level) and few cases in the mountains. Most of our population is an ethnic admixture of Europeans (Spaniards colonists), Africans (slaves), and Amerindians. The purpose of our study was to determine the origin of the β^s globin haplotype, age and survival dependency, and the admixture among the different African groups in our population. The $\alpha^{3.7}$ globin gene deletion status was also studied and found in a very high frequency. DNA from peripheral blood of 191 non-related patients (81 with HbS homozygous and 15 patients compound heterozygous for HbS, HbC, HbD with β-thalassemia, and 95 with sickle cell trait) were studied. The β^s chromosome was linked 51% to the Benin Haplotype, 29.5% with the CAR, 12.5% to the Senegal, and 2.5% to the Cameroon. We did not find any significant difference between the haplotype distribution among adults and children and among sickle cell patients and traits. Only 8.6% of the patients have homozygosity for the Benin haplotype. These results show a very high frequency of admixture in our African origin population. Am. J. Hematol. 64: 87–90, 2000. © 2000 Wiley-Liss, Inc.

Key words: β-globin haplotypes; sickle cell anemia; α-thalassemia

INTRODUCTION

The presence of Hb S has been known in Venezuela for 50 years, and it presents a variable distribution with a high frequency in the coastal area (sea level) and seldom cases in the mountains. Venezuela is located at the North of South America and it has a large coast in the Caribbean Sea. Most of our population is an ethnic admixture of Europeans (Spaniards colonists), Africans (slaves), Amerindians, and later on the Italian, Spaniards, and Portuguese immigrants [1]. The β^s globin has a variable presentation, in population surveys it has been found to have a frequency as high as 19%, in isolated coastal communities, and as low as 0.0% in the Andean regions [1–3].

In a small Venezuelan village, the β globin gene cluster haplotypes were studied in normal population. The Benin haplotype represented 20.9% among 12 haplotypes studied, which indicates an important contribution of an African ancestry in that population [4]. In a previous study of a smaller group of sickle cell anemia patients the most prevalent linkage of the β^s chromosome was to the Benin haplotype [5].

We wanted to determine the origin of the β^s globin gene in our sickle cell anemia (SCA) patients as well as the age dependency, as has been noted in Cuba and Guadeloupe [6,7]. In order to avoid distortions in the frequency of any specific group, we also studied the β gene cluster haplotypes among sickle cell traits. We explored the frequency of α-thalassemia and if it had age dependency.

SUBJECTS AND METHODS

The study was performed in 191 non-related patients proceeding from all over the country, under regular fol-

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TABLE I. Distribution of β^s Haplotypes in SCA and Sickle Trait (HbAS)*

	SCA		HbAS	
β^s haplotypes	No.	%	No.	%
Benin	90	50.8	49	51.6
CAR	57	32.2	24	25.3
Senegal	25	14.2	9	9.4
Cameroon	4	2.3	3	3.2
Atypical	1	0.5	10	10.5
Total	177		95	

*No significant difference ($\chi^2 = 5.3$, $P = 0.1499$) was found between SCA and sickle trait patients.

TABLE II. Distribution by Age of β^s Globin Gene Haplotypes in SCA Patients*

	Adults ($n = 43$)		Children ($n = 53$)	
β^s haplotypes	No.	%	No.	%
Benin	45	52.9	45	48.9
CAR	25	29.4	32	34.7
Senegal	12	14.2	13	14.2
Cameroon	3	3.5	1	1.1
Atypical			1	1.1
Total	85		92	

*No statistically significant difference ($\chi^2 = 1.3$, $P = 0.5226$) was found between age group.

low-up in the Hemoglobinopathy Clinic of the Hospital Universitario de Caracas and the Hospital Civil de Maracay; 81 of them with Hb S homozygous, 15 compound heterozygous with Hb C, Hb D, and β-thalassemia, and 95 with the sickle cell trait. All patients and their parents provided consent to participate in this study. Phenotypes were identified by hemoglobin electrophoresis in acetate and citrate agar, solubility tests, and family studies. Diagnosis of β-thalassemia was confirmed by mutation identification using ARMS-PCR [8]. DNA from peripheral blood leukocytes was prepared as previously described [9]. Seven fragments of the β-like globin gene cluster were amplified by PCR using the primers described by Sutton and co-workers [10]. They were digested with restriction endonuclease to identify the polymorphic sites *Xmn*l 5′ to the $^{G}\gamma$ gene, *Hin*dIII in the IVSII of $^{G}\gamma$ and $^{A}\gamma$ gene, *Hinc*II 3′ and inside the $\phi\beta$ gene, and *Hin*fI, *Hpa*I, respectively, at 5′ and 3′ of the β gene. Using these seven polymorphisms, the chromosomes were grouped in 5 haplotypes: CAR (−+−−−−+), Benin (−−−−+−−), Senegal (++−−+++), Cameroon (−++−+−+), and Atypical haplotypes (all other combinations). The $-\alpha^{3.7}$ thalassemia cases were detected by GAP-PCR [11].

RESULTS

The haplotypes of β^s chromosomes determined in 191 patients (272 chromosome) is shown in Table I. Distribution of the 177 chromosomes from the SCA patients studied was as follows: Benin haplotypes (Ben) 50.8%, followed by Bantu (CAR) 32.2%, Senegal (Sen) 14.1%, Cameroon (Cam) 2.3%, and atypical haplotypes (Aty) 0.5%. In the 95 sickle cell trait patients the distribution of the β^s gene was as follows: Ben 51.6%, followed by CAR 25.3%, Sen 9.4%, Cam 3.2%, and Aty 10.5%. The frequency distribution of the haplotypes was compared between SCA and HbAS, and no statistical difference (χ^2 5.3, $P = 0.1499$) was noted among both groups. This was done in order to avoid a variable due to early death in a specific group. When age was considered, we found no statistical difference in the different age groups (χ^2 1.3, $P = 0.5226$; Table II). We found a very high frequency of mixed haplotypes 82%, and only 8.6% were homozygous for Benin haplotype.

The −29 β thalassemia mutation was the most prevalent among the compound heterozygous Hb S-β thalassemia patients.

In 148 patients we found 92 (62.2%) with both α genes normal, 48 (32.4%) heterozygous for $-\alpha^{3.7}$ thalassemia, and 8 (5.4%) were homozygous for the same deletion. When age distribution was studied among the patients, with one or both α genes deleted, no difference between age groups was found (Table III). There is a very high frequency of $-\alpha^{3.7}$ thalassemia (0.27) in the 296 patients studied.

DISCUSSION

The β^s mutation may be associated with at least four different major β^s haplotypes, named according to the geographic areas where it predominates: the Benin, the Senegal, the Central African Republic, and the Asian types [12,13]. Historically it is known that some slave vessels came straight from Africa to Venezuela. Afterward slaves were brought over as a result of licit commercial transactions from the Antilles for agriculture and mining works. Runaway slaves from Curacao and Trinidad arrived in Venezuela surreptitiously remaining free in the jungle, they were very aggressive and called "Black Cimarrons." Later they formed the population we find currently in the mountains. From an anthropologic point of view it is certain that the African population arriving in Venezuela possibly constituted a heterogeneous group which proceeded apparently from well-delimited African regions, Sudan and Bantu [14]. The studies of the β^s gene cluster are useful in the definition of the origin of the population brought by the slave trades. The incidence of β^s haplotypes in our country could be different according to the region studied as we know the frequency of HbS is variable and spread all over the country, been stronger in populations stemming from around the sites where the African first resided.

TABLE III. α Thalassemia Frequency in Patients from Cuba, Guadeloupe, and Venezuela According to Age Group*

	Cuba		Guadeloupe		Venezuela	
Age	Frequency	No.	Frequency	No.	Frequency	No.
0–20	0.14	74	0.18	169	0.30	64
21–50	0.19	144	0.2	110	0.23	32
>50	0.34	16	0.2	10	0.37	4
Total		234		289		100

*In the Guadeloupean and Venezuelan groups no statistically significant difference was found. In the Cuban group there was a significant difference between the age groups.

TABLE IV. Distribution of β^s Haplotypes in American Countries*

β^s haplotypes	Brazil	Cuba	Guadeloupe	Jamaica	EEUU	Venezuela[a]
Benin	25	51	74	72	62	51
CAR	73	41	11	17	17	32
Senegal	1	8	6	10	10	14

*Data from Zago [16], Muñiz [5], Kéclard [6], Antonarakis [14], and Nagel [19].
[a]Report from present study.

In our study, we found the Benin β^s haplotype as the most prevalent form, same as was found in Cuba, Guadeloupe, United States, and Jamaica [5,6,15,16], followed by the CAR haplotype, the most prevalent one in Brazil [17,18] (Table IV). Our findings were to be expected on the basis of African ancestry according to Curtin et al. [19], Nagel [16], and Pagnier [12]. These slaves had come from Central West African ports. The African Negroes brought to Brazil, monopoly of the Portuguese, were mostly from Angola, Congo, and Mozambique [19] where the CAR haplotype predominates. We found a very high frequency of mixed β^s haplotypes in 82% of our patients, as was expected due to the high ethnic admixture.

The prevalence of $-\alpha^{3.7}$ thalassemia in 37.8% of the subjects, with one or both chromosomes deleted, is one of the highest reported. This finding agrees with a previous report of a smaller group of patients [20]. No statistically significant difference was observed between children and adult with $-\alpha^{3.7}$ thalassemia.

The studies by Nagel [21], Kusolik [22], Powars [23], Steinberg [24,25], and Chang [26] have demonstrated that the different β^s gene cluster polymorphism modulates the clinical severity of the disease. The haplotypes and gender interact to modify the hematological parameters. They also may explain the phenotypic heterogeneity among the patients. As most of our patients of the Ben/CAR mixed haplotype, they should have the same clinical course, "benign/worst," which is not what we see in our clinical practice. Even siblings with the same mixed haplotypes, with $-\alpha^{3.7}$ thalassemia, the same environment, and socioeconomic conditions have different clinical behavior. This is one of the reasons for our belief that the problem has not been solved and that there must be other genetic variables modulating the clinical expression of our patients.

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